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The Pineal Gland, via Melatonin, Protects DNA, Coordinates the Endocrine System with the Immune System and Controls the Timing of Reproduction

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The Pineal Gland, via Melatonin, Protects DNA, Coordinates the Endocrine System with the Immune System and Controls the Timing of Reproduction

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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TABLE OF CONTENTS

Page

LIST OF FIGURES....iv

LIST OF ABBREVIATIONS....v

INTRODUCTION....1

A FREE RADICAL SCAVENGER....9

ENDOCRINE SYSTEM AND REPRODUCTION....15

TACHYKININS....29

IMMUNE SYSTEM....37

CANCER....42

STRESS AND DISEASE....51

CONCLUSION....56

LITERATURE CITED....59

VITA....73

LIST OF FIGURES

Figure Page

1.....69

2.....70

3.....71

4.....72

LIST OF ABBREVIATIONS

HIOMT- Hydroxyindole-o-methyltransferase
aMT6- 6-sulfatoxymelatonin
SCN- suprachiasmatic nucleus
SP- substance P
NKA- neurokinin A
SCG- superior cervical ganglia
CNS- central nervous system
CSF- cerebrospinal fluid
ALA- delta-Aminolevulinic acid
TSH- thyroid stimulating hormone
LH- luteinizing hormone
GH- growth hormone
FSH- follicle stimulating hormone
GnRH- gonadotropin releasing hormone
ADH- antidiuretic hormone
PRL- prolactin
LHRH- luteinizing hormone releasing hormone
ME- median eminence
NPK- neuropeptide K
NPG- neuropeptide *y*
POMC- proopiomelanocortin
VIP- vasoactive intestinal peptide
TRH- thyrotropin releasing hormone
PSA- prostate specific antigen test
TGF- transforming growth factor
IL-2- interleukin 2
TNF- tumor necrosis factor
GM-CFU- granulocyte/macrophage colony forming units
CSF- colony stimulating factors
S-CFU- spleen colony forming units
HPA- hypothalamic-pituitary-adrenal
ACTH- adrenocorticotropic hormone
AIP- acure intermittent porphyria
Ala- delta-aminobutyric acid
GABA- gamma aminobutyric acid

ABSTRACT

THE PINEAL GLAND, VIA MELATONIN, PROTECTS DNA, COORDINATES THE ENDOCRINE SYSTEM WITH THE IMMUNE SYSTEM AND CONTROLS THE TIMING OF REPRODUCTION

By Craig Lincoln Anthony, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2001

Director: Dr. Hugo Seibel

The pineal gland secretes the hormone melatonin (n-acetyl-5-methoxytryptamine) with a circadian rhythm. This secretion's rhythm becomes disrupted with age. When melatonin secretion is decreased with advanced age, the immune, endocrine and reproductive systems fail to function optimally. Melatonin possesses lipophilic and anti-oxidant properties, providing it with access to nuclei. Melatonin protects the DNA, preventing cancerous mutation. Hippocampal degeneration and age increase and prolong the adrenocortical responses to stress. Melatonin supplementation reduces the prolonged exposure to harmful hormones.

INTRODUCTION

All organisms function according to a biological rhythm, usually circadian, from the subcellular level through the organismal level (130). These rhythms generally become increasingly disrupted with age. Recently, scientific debate has speculated that these disruptions of rhythm may be the result of ageing. They could also be a significant contributor to ageing. With the discovery of melatonin as the hormone directly related to these rhythms, the query whether these disturbances are causing ageing or are the result of ageing has received increasing interest. This study is less concerned with preventing ageing and more interested in melatonin's ability to maintain a more youthful homeostasis with increasing age through supplementation, possibly via maintaining hormone and system balance, possibly via maintaining youthful hormonal concentrations and homeostasis. Indirectly, this supplementation may affect the longevity of an organism by retaining the environmental plasticity seen in youth.

The pineal gland secretes the hormone melatonin (n-acetyl-5-methoxytryptamine). Melatonin is secreted with a circadian rhythm and a plasma concentration peak 3 to 10 times higher than base levels at approximately 3 a.m. Melatonin, produced and secreted without significant storage, is the chemical signal relaying circadian rhythms to almost every cell in the body. Melatonin's lipophilicity provides it broad access to cellular cytoplasm, nuclei and multiple membrane receptor locations. Melatonin is produced in the pineal gland from n-acetylserotonin. N-acetylserotonin is converted from serotonin by n-acetyltransferase, the rate limiting enzyme, and converted into melatonin by hydroxyindole-o-methyltransferase (HIOMT). The two step degradation consists of a 6-hydroxylation by microsomal monoxygenases followed by conjugation from a cytosolic

sulfotransferase. This produces 6-sulfatoxymelatonin (aMT6), the main metabolite, in 70-80% of degraded melatonin (124). Minor quantities of melatonin (10-20%) are conjugated to glucuronic acid [Fig. 1]. The hydroxylation that occurs in the liver is inducible by phenobarbital and polyaromatic hydrocarbons (124). This secretion is consistent regardless of species and diurnal or nocturnal activity. Thus, melatonin is recognized as an internal synchronizer of cosmic chronology.

The pineal gland, epiphysis cerebri, is a small cone-shaped structure suspended from the caudal epithalamus superior and caudal to the midbrain. The pineal gland contains three main cell types; calcareous granules, astrocyte resembling glial cells and parenchymal cells. The parenchymal cells extend into the basal lamina of the perivascular space (82). The pineal gland is well vascularized with fenestrated capillaries and receives only one innervation. These postganglionic sympathetic nerves arrive at the pineal gland from the superior cervical ganglia. This is the final step of a complicated pathway that conveys environmental photoperiod information from the retina to the pineal gland. This retinal photostimulation is relayed to the supra-chiasmatic nucleus (SCN). From the hypothalamus, the nerve signal is then sent to the upper thoracic intermediolateral cell columns. Preganglionic sympathetic neurons exit through the white rami, reach the sympathetic trunks and finally the superior cervical ganglia. The final step in the regulatory pathway of melatonin is the neurotransmitters released from noradrenergic terminals that originate in the superior cervical ganglia (80), targeting the pineal gland. This complicated pathway of innervation may be the result of a long evolutionary history of the mammalian pineal gland.

Another regulatory pathway of melatonin is not directly linked to light/dark modulations. Tachykinins may regulate pineal function, independent from environmental light (19). Substance P (SP) and neurokinin A (NKA) are either released from nerve fibers not originating in the superior cervical ganglia (SCG) or are produced in the pineal gland.

The exact origin is not known conclusively, although it is speculated that they are produced in the pineal gland. Removing the SCN did not result in a change in concentration of SP in the pineal gland (111). Research looking for the gene and mRNA encoding the synthesis of preprotachkinin peptides in the nucleus of pineal gland cells is currently being explored to definitively prove that tachykinins are produced in the pineal gland. Neuropeptide K and neuropeptide gama are also suspected to be present, as both are co-synthesized with SP within preprotachykinin peptides. HPLC experiments revealed an inconclusive, immunoreactive peak of similar weight to neuropeptide K (19).

Unlike melatonin and the noradrenergic neurotransmitters, NKA did not change concentration in the pineal gland with environmental light (19). Pineal NKA concentrations were no different in rats killed during light or dark periods. Tachykinins do not express circadian rhythms, although they are sensitive to endocrine regulation. Gonadotropins and gonadal steroids modulate the concentrations of tachykinins in the pineal gland, while melatonin has no direct affect (19). Pineal tachykinins increased in concentration following castration. The castrated animals' pineal NKA concentrations returned to normal with testosterone replacement therapy (19). Testosterone has an inhibitory effect on pineal tachykinin production (19). Superior cervical ganglionectomy also resulted in an increase in pineal tachykinin concentrations. Although pineal tachykinins are not released by the noradrenergic nerve terminals originating in the SCN, the neurotransmitters that are released by these terminals inhibit pineal tachykinin production.

The manner of regulation the tachykinins exert on the pineal gland is unknown. SP receptors have been found in the pineal gland. SP has been found to increase adenylate cyclase activity in the pineal gland, and the other tachykinins have shown stronger affects than SP in the salivary gland (123). Tachykinins may be transported to the pineal gland or synthesized in situ. They are regulated by androgen concentrations and noradrenergic

nerves originating in the SCN. Although both of these forms of regulation follow circadian rhythms, tachykinin concentrations in the pineal gland do not .

Tachykinin, NKA, concentrations in the hypothalamus (39) and anterior pituitary (22) increase when exposed to testosterone and decrease with the loss of testosterone. This is the opposite effect seen in the pineal gland. The tachykinins role in the complicated regulation of melatonin production is worthy of more research. Tachykinins possess the unique quality, with regard to melatonin regulation, of being independent of circadian rhythm. Estradiol and progesterone affect melatonin secretion. Norepinephrine synthesis is modified by gonadal hormones in the sympathetic nerves originating in the SCN and terminating at the pineal gland (106). Therefore, it is logical that gonadal hormones affect tachykinins to some extent, and tachykinins help modulate melatonin regulation with contributions to this indirect relationship with circadian rhythms.

One of the crucial elements for the evolution of life, the sun, displays rhythms of about 24-hour duration. These are circadian rhythms. Due to the importance of this rhythm, melatonin has been conserved from species to species.

At its most basic level, evolution is the plasticity a species expresses as a result of changes in its environment making the organism more successful. The earth's revolution around the sun is the largest and most consistent influence on the earth's environment. It contributes to almost every organisms' development on the planet. Therefore, it is not unexpected that not only has melatonin developed at an early stage of evolution, but it has remained unchanged throughout this development. Organisms have evolved multiple uses for this constant in their biology.

If melatonin proves to be one of the earliest free radical scavengers, this would almost certainly define the evolutionary origin of melatonin. Melatonin's protection against free radical damage could have led to other uses in organisms due to its photoperiodic responses and photooxidation with light (46). Melatonin functioned as a

dark mediator, even at the unicellular organism level. Melatonin conveyed photoperiodic and temperature information to the unicellular organism *Gonyaulax* (46). In *Gonyaulax*, melatonin levels are highest in dark and low temperature environments (15 degrees C) (46). More evolved organisms retained these functions of melatonin while finding additional uses for the molecule. Melatonin remains a valuable free radical scavenger in areas exposed to high levels of hydroxyl radicals and structures requiring additional protection, e.g., CNS and DNA.

Humans, one of the most evolutionarily advanced species on the planet, have developed many uses for the consistency of melatonin secretion. Melatonin is found throughout the body and has access everywhere. Melatonin is mainly produced in the pineal gland, but also in the retina and GI tract. Two other consistencies in human melatonin production are the calcification of the pineal gland with age and the steady decline of melatonin production with age after reproductive viability has expired.

The calcification of the pineal gland is not related to production of melatonin as it does not change the histology of the pinealocyte (132) or HIOMT activity, the enzyme responsible for facilitating melatonin production (137). Increased excretion of melatonin with age is also unlikely to be the cause of lowered plasma concentrations as urinary 6-hydroxymelatonin, melatonin's main metabolite, is reduced proportionally (71).

The sympathetic innervation of the pineal gland may be the cause of decreased melatonin production with age. Pinealocytes have *B*-adrenergic, membrane bound receptors that respond to norepinephrine by stimulating melatonin synthesis. Studies in rodents have shown both a decrease in *B*-adrenergic receptors and receptor sensitivity with age (41). This is, currently, the most probable explanation for decreased melatonin synthesis and plasma concentrations in aged humans.

Decrease in melatonin synthesis may also be due to altered physiology in the brain causing a disruption in yearly circadian rhythm, rather than daily circadian rhythm. As

melatonin is produced at night, melatonin production increases during winter months with the shortest photoperiod. Other studies have shown peak melatonin production to exist in both summer and winter with lowest levels in spring and fall (7). In some young, healthy male individuals, melatonin production is highest in the summer months (130). Other studies have found no seasonal variations in melatonin plasma concentrations or no seasonal variations in concentration, but a phase advance of about ninety minutes during winter months (53). These variations in results suggest a complicated process of converting photoperiod to melatonin production. A consistency in study results is the summer months peak of melatonin in healthy, young males. The variations suggest further brain activity, regulating melatonin synthesis. This processing may occur in the SCN before a sympathetic nerve signal is sent to the pineal for melatonin synthesis. Additional research is necessary to determine if the alterations in seasonal melatonin production are correlated to reproductive success, different individuals hormonal balance or simply latitude.

Aged patients show a decrease in melatonin production with a minimum plasma concentration in October (130). Alzheimer patients' melatonin production is further depressed, also with a minimum concentration in October. In both cases, the decrease in melatonin secretion is accompanied by an alteration of the seasonal variability of melatonin secretion. The phase shift in seasonal variability of secretion would more likely result from alterations or diminished brain processing of photoperiod signals than decreased number or sensitivity of *B*-adrenergic receptors.

With the complexity of photoperiod information processing and delivery to the pineal gland, it is unlikely the disturbance in rhythm seen with age is isolated to one area of the brain. The SCN would be the first area to investigate. Telencephalon and cerebrum, areas involved in Alzheimer's disease, could also be involved, due to the correlation between extremely low melatonin secretion and the disease. Diencephalon structures

associated with sensory information or homeostasis, thalamus, hypothalamus, may also be involved, due to their common embryonic development and interaction with the epithalamus, pineal gland.

As melatonin's regulator in the human brain, the supra-chiasmatic nucleus has been dubbed the internal pacemaker. Retinal signals are transmitted to the SCN. These retinal signals are a result of environmental photoperiods. The SCN transmits neural inputs to the pineal gland. The pineal gland responds by producing and secreting melatonin in a circadian fashion. The timing and expression of seasonal and daily light regulate circadian rhythms crucial to every system in the body, most notably reproduction and the equilibrium present between the endocrine and immune systems. Melatonin is a lipophilic molecule, allowing it access to almost every cell in the body. Circadian regulation of the reproductive system is crucial for seasonal breeders. Melatonin receptors are more prevalent and express a lower affinity in birds than mammals (16). This may allow for more precise regulation from melatonin. Birds produce melatonin as a result of light illumination through their skull, as well as retinal signaling. Birds may have evolved with additional uses for melatonin. They may use melatonin for migrational navigation and timing.

In humans, melatonin increases in production and secretion nocturnally, with a peak concentration between two and three a.m. As an organism ages, a peak of melatonin production is seen prior to puberty and gradually declines until death. The initial decline in melatonin plasma concentration is due to the expansion of the vascular system with increased organism size. This decline initiates puberty through melatonin's decreased inhibition of the endocrine system, specifically gonadotropins and androgens. The gradual decline in melatonin production with age is not caused by the calcification of the pineal gland. Melatonin production decreases as a result of reduced adrenergic innervation of the pineal gland and a decrease in the concentration of *B*-adrenergic receptors on the

membranes of pinealocytes (108). Melatonin is primarily produced in the pineal gland under the influence of the SCN, although melatonin is also produced in retinal cells and enterochromaffin cells from the GI tract to a lesser extent.

Circadian rhythms have been known to influence the ageing of species for decades. In 1972, Pittendrigh and Minis shortened the life span of *Drosophila melanogaster* by transplanting flies raised on a 24 hour cycle to either a 21 hour or 27 hour cycle (117). These rhythms are primarily controlled by the SCN in humans. The SCN does not exhibit morphological changes with age, but rather changes in its function (105). Body temperature is regulated by the SCN. Body temperature has a strong influence on sleep-wake cycles. Sleep disturbances are among the chief medical complaints in older patients (105).

A FREE RADICAL SCAVENGER

Melatonin is intimately involved in the regulation of the immune and reproductive systems. It fights cancer, facilitates pregnancy and creates a hormonal homeostasis. Yet, melatonin's evolutionary value may result from one necessity for survival of every organism, protecting the DNA from free radical damage.

Melatonin's original development as a free radical scavenger could be solidified if a correlation was found between melatonin's phylogenic age and the development of aerobic respiration. Aerobic respiration consumes nearly all cellular oxygen. The 1-2% of oxygen that is not completely reduced may form either a superoxide anion radical or hydrogen peroxide (126). Neither molecule is as cytotoxic as hydroxide radicals, but hydrogen peroxide can rapidly be converted into the hydroxide radical in the presence of redox-active transition metals through the Fenton reaction. Melatonin's effectiveness as a free radical scavenger with a specific affinity to hydroxide radicals was compared to mannitol and glutathione, two strong free radical scavengers (125). This experiment discovered that melatonin was as effective a scavenger of hydroxide radicals as glutathione and mannitol at one sixth and one tenth the concentration, respectively.

Melatonin has been found in almost every species in the animal kingdom. Melatonin was originally thought to be exclusively produced by the pineal gland. With the discovery of melatonin production in unicellular organisms, e.g., the dinofagellate *Gonyaulax polyedra*, it was realized that melatonin is also produced by other cells than pinealocytes.

If melatonin is found to be an early free radical scavenger, this would explain melatonin's affinity, bordering on specificity, to hydroxide radicals, the most destructive

free radicals. Melatonin's high lipophilicity is additional evidence supporting melatonin's original development as a free radical scavenger. The first free radical scavenger would certainly evolve to protect organisms' DNA. Melatonin is the most well designed free radical scavenger for this task.

An experiment using safrole, a carcinogen found in sassagras oil, supported claims of melatonin's role in DNA protection (126). Safrole induces oxidative radical attack of DNA, thus promoting cancer. A low dose of melatonin (.2 mg/kg) was administered to rats prior to a safrole treatment. This pretreatment reduced safrole's damage of hepatic DNA by over 40%. With a higher dosage pre-treatment (.4 mg/kg), melatonin reduced safrole's damage of DNA to within 1% of the control.

As these results are more significant regarding the prevention of cancer, rather than the treatment, another experiment was designed to determine melatonin's protection of DNA at physiological concentrations (126). Safrole was injected into rats during the day (low plasma concentrations of melatonin) and at night (peak plasma concentrations of melatonin). Safrole damage to DNA was reduced by over 20% when injected at night, when compared to day injections.

Melatonin may have evolved for the protection of DNA from free radical damage, although other forms of protection have been attributed to this hormone. Oxygen-centered radicals can suppress the calcium pump in cardiomyoctes. Melatonin protects rat cardiac sarcolemmas from these free radicals (126). Melatonin's peripheral protection against free radicals would have evolved after its ability to protect nuclear DNA, or would have been a coincidence of similar molecular design requirements. In either case, other free radical scavengers would later evolve to help protect a wider assortment of biomolecules throughout the organism.

Reiter proposed the mutual evolution of aerobic respiration and melatonin for protection against the free radicals aerobic respiration produces (126). He used the

physicochemical properties of melatonin to support the use of membrane-bound receptors for melatonin. These receptors are required for the more evolutionarily advanced functions of melatonin, including circadian rhythm regulation, hormonal balance, coordination of immune and reproductive systems and possibly the regulation of metabolism in hibernating organisms.

The addition of membrane receptors reflects organisms' evolution to increase the uses of melatonin. Melatonin has not changed in chemical structure throughout evolutionary time (126). The dinoflagellate, *Gonyaulax polyedra*, not only produces melatonin, but it cycles with a circadian rhythm, as seen in humans (107). The membrane receptors increase the potential effects of melatonin, but are yet to be widely distributed (126).

The actions of melatonin through receptors, combined with melatonin's lipophilicity, give this molecule the diversity to affect almost every cell in the body. Melatonin can transverse the blood brain barrier (47), or travel in CSF. Melatonin is non-toxic at high plasma concentrations. Reiter was unable to produce toxic side effects or prooxidant activity with high melatonin doses chronically administered (126). Being non-toxic at high concentrations, melatonin can greatly vary its physiological plasma concentrations with circadian rhythm, and it can easily be used as a clinical treatment or as preventive medication. Melatonin can readily be enzymatically degraded in the liver with retinal exposure to light acting as an initiator.

Melatonin's main target is the nucleus. Its protection of DNA from free radical damage is valuable, but this nuclear access has other benefits. Melatonin may be bound in the nucleus and could directly affect gene transcription (78). Dr. Reiter has shown that melatonin intercalates with DNA (126). Melatonin is found in higher concentrations in the nucleus than the cytosol (78). This puts melatonin in a more direct position to influence an organism and allow the organism to evolve tailored uses for such an ubiquitous molecule.

Melatonin is produced in a variety of tissues throughout the human body. Most tissues that produce melatonin also contain porphyrins producing high levels of free radicals (126). Circadian rhythmic melatonin production occurs in phylogenetically old, single celled organisms (107). The pineal gland does not appear crucial to the prevention of free radical damage or circadian rhythm in all species. Humans may have evolved a pineal gland as our systems became more complex. The pineal gland, devoted exclusively to melatonin production, may allow a more precise production and feedback of melatonin. This refinement of the use of melatonin may have allowed for another use. Melatonin may be the protector of DNA, allowing humans to reach reproductive maturity and increase the statistical chance of reproduction in such a complicated organism. The pineal gland begins to calcify and melatonin production diminishes as reproductive potential decreases. Free radical damage, DNA damage, disease and cancer rates increase.

Rat lung and spleen tissue have been protected from oxidative damage to DNA by melatonin. In this respect, melatonin acts as an anti-oxidant, possibly scavenging free radicals. It is particularly well suited for protecting nuclear DNA due to its small size and lipophilicity.

Reiter infected rats with the potential carcinogen, delta-Aminolevulinic acid (ALA)(106). These rats showed higher levels of nuclear DNA and membrane oxidation in lung and spleen tissue than the controls. When melatonin was injected following the ALA injection, the test group's oxidation levels were similar to the control group's levels. Melatonin protects DNA and lipid membranes from oxidation by detoxifying OH and NO, scavenging H2O2 and stimulating other anti-oxidative enzyme activity(106).

Specifically, melatonin does not trap free radicals. It inhibits metal ion-catalyzed oxidation of these free radicals. Melatonin is precluded from the group of antioxidants that trap free radicals because it is a simple, substituted indole structure(100) This type of

structure does not contain a removable hydrogen which is necessary and available in the phenoxyl position of alpha-tocopherol and other antioxidants.

Melatonin acts as a preventive antioxidant by forming a bond between its ring nitrogen and the metal ion. This inhibits the catalyzation of oxidation. This type of antioxidant is not a scavenger, it is a preventive antioxidant. This antioxidant exhibits wider influences on the control of free radicals in the body than a scavenger, such as vitamin E.

Other, in vitro, studies have shown melatonin to exhibit twice the free radical protection than vitamin E(87). These authors readily admit that they do not have reproducible data supporting such results in vivo, but the in vitro experimental results are impressive.

Melatonin undoubtedly shows multiple antioxidant properties, but these actions are not its primary function. Melatonin is a genetically, highly conserved molecule. Its main role, as an antioxidant, is to protect DNA.

OH free radicals are a primary destroyer of DNA. Due to their structure, most free radical scavengers are not capable of crossing the cytosol and entering the nucleus of a cell. Even if free radical scavengers could exist in close proximity to DNA, the typical hydroxyl radical inflicts its damage on the genetic material before it migrates one or two of its molecular diameters(106). This leaves insufficient time to scavenge all these free radicals considering the dire consequences if one comes in contact with the DNA. One reason melatonin may be highly conserved, genetically, could be to protect DNA from free radicals by inhibiting their formation. Melatonin's structure allows access to the nucleus and therefore the DNA, and its mechanism of preventing free radical damage is preemptive rather than reactive. These are traits valuable to the protection of DNA.

Melatonin may have evolved initially to protect DNA from free radical damage. It could then have developed into a more general antioxidant. As organisms became more

complex, melatonin receptors allowed more diverse actions. Humans have developed multiple complex uses for melatonin. One of the most complex actions of this tropic hormone is maintaining the appropriate balances of almost every other hormone in the endocrine system.

ENDOCRINE SYSTEM AND REPRODUCTION

The pineal-hypothalamic-pituitary axis is complex and its deterioration with age is not completely understood. Biochemical and physiological responses to hormones decrease with age. Most steroid receptors do not lose affinity for their hormones, but reduce in concentration with age. *B*-adrenergic receptors form an activated complex with adenylate cyclase. This complex, specifically, exhibits a decline in affinity for norepinephrine with age (127). During the night, there is an increase in adrenergic input to the pineal gland through the sympathetic nervous system (127). The concentration of *B*-adrenergic receptors increases on the pineal gland with light exposure (127). As the pineal gland ages, it is unable to accommodate these necessary changes in receptor density and sensitivity to support normal melatonin secretions. Norepinephrine has less affect on the pineal gland in aged individuals. Melatonin secretion is decreased at night, as it is mediated by norepinephrine through the *B*-adrenergic receptors. The calcifications occurring in pineal glands with age are structural changes and do not appear to be the cause of decreased nocturnal melatonin secretion with age (35).

Melatonin, TSH, prolactin and GH amplitudes are depleted with age (13). LH and FSH levels are elevated. Testosterone secretion is decreased. Cortisol loses some rhythm, but is changed little with age.

LH concentrations begin to rise in older men as the testosterone levels begin to decrease. Without adequate levels of testosterone inhibition, LH levels are elevated. Aldosterone and prolactin are secreted rhythmically, with a peak at night. Both exhibit a decrease in this circadian amplitude with age.

A decrease in gonadal activity with age results in a compensatory rise in gonadotropin secretion. As the high concentrations of plasma LH are compatible with its circannual rhythm, the rhythmic hormonal secretion of the pituitary gland is independent of decreased gonadal hormone secretion and concentration in aged individuals (131). The rhythmic pituitary secretion is not degraded by the loss of gonadal hormones. This is true of both testosterone, which decreases with age, and estradiol and progesterone, which decrease after menopause.

The pineal gland is intimately coordinated with the activities of the hypothalamus, median eminence and pituitary gland. Thus, it is not surprising that melatonin helps regulate release of gonadotropins (LH and FSH), prolactin and GH.

The pituitary stores and secretes gonadotropins and prolactin. The in vivo addition of melatonin has no affect on LH or prolactin storage in the pituitary (2). Melatonin produces an inhibitory influence on high levels of prolactin and gonadotropin secretion in the young(110). Thus, the highest concentrations of melatonin exist before puberty. The drop in melatonin promotes puberty by increasing gonadotropin secretion. Melatonin's affect on secretion and not storage suggest different regulatory mechanisms for each and the lack of influence melatonin exhibits on the biosynthetic pathway.(3) Pituitary tissue stores LH for future surges, such as inducing ovulation or puberty. Melatonin has been analyzed as a potential treatment to delay reproductive senescence.(76) This line of research logically followed the discovery of melatonin's ability to delay puberty by inhibiting prolactin and gonadotropin secretory surges.(30) The functional capacities, secretion of hormones, of the pituitary gland and the median eminence can be maintained and extended through reproductive senescence with melatonin supplements. When the anterior pituitary was transplanted from acyclic, senescent rats to cyclic, young rats with the cyclic rats anterior pituitary removed, the cyclic rats pituitary could not maintain normal estrous cycles.(110) There was a decrease in GnRH receptor numbers. This

decrease in receptor concentration is hypothesized to be the reason acyclic anterior pituitary glands can not maintain normal estrous cycles in cyclic rats. In youth, melatonin inhibits the pituitary's response to GnRH.(122) Contrarily, melatonin supplements, in aged females, increase GnRH mRNA production by 18%, returning GnRH levels to those of youth.(64) However, the functional status of the hypothalamic-pituitary system in senescence is still not well understood and is in need of further research to solidify existing theories.

Although some of the mechanisms are not understood, many hormonal reactions to melatonin supplements are documented. When melatonin concentration is increased in young, cyclic rats, with high levels of gonadotropins, the gonadotropin secretion is decreased to lower levels.(3) The pituitary LH concentrations did not change and, although acyclic mammals secrete more gonadotropins than cyclic mammals, the pituitary tissue content remains constant. This could be a result of different, age dependent, selective factors regulating gonadotropin release from the pituitary gland;(3) different neuroendocrine mechanisms controlling the storage of gonadotropins in the pituitary tissue;(85) or alterations of hypothalamic-pituitary axis function relative to age(52). Regardless of the mechanism, melatonin's inhibitory affects are exerted on the secretory process rather than pituitary storage.

The median eminence secretes prolactin and gonadatropins, primarily FSH. As in the pituitary, melatonin decreases prolactin and gonadotrophic release, in cyclic mammals, from the median eminence.(3) Similar to the pituitary, the median eminence tissue content of LH changes little with age, although FSH content in the tissue of the median eminence decreases with age. FSH and LH secretions from the median eminence increase as the mammal becomes acyclic. The relative gonadotropin secretion from the median eminence is minor compared with the secretion from the anterior pituitary.

Melatonin does not change the tissue content of LH from the median eminence, nor the pituitary in cyclic or acyclic mammals(3). Melatonin decreases the pituitary LH secretion in cyclic animals. Melatonin does not change the LH secretion from the median eminence in cyclic mammals, as there is not significant LH secretion from the median eminence.

The median eminence is more significant with regard to FSH, compared with other median eminence hormones. FSH is secreted and stored in similar concentrations in both the pituitary gland and the median eminence. Ageing increases both tissue storage and secretion of FSH, in the pituitary gland and median eminence. When melatonin is administered to cyclic mammals, median eminence and pituitary FSH secretion and tissue storage concentrations are lowered. Acyclic mammals respond to melatonin by decreasing FSH storage and secretion(3). Although the median eminence has much larger concentrations of FSH than LH, the pituitary gland is primarily responsible for gonadotropin storage and secretion. Inhibin, a hormone produced by granulosa cells in atral follicles, declines in concentration as follicle cells are lost with age and menopause. Inhibin inhibits FSH secretion from the pituitary. With the loss of this inhibition, FSH plasma concentrations rise with age.

Melatonin secretion varies with seasons, as well as day or night. Although the results vary based on lattitude, generally, the highest basal melatonin concentrations occur in February, and the lowest concentrations occur in Novermber(9). This corresponds with the shortest and longest sunlight exposure per day. February is two months after the winter solstice, and November is five months after the summer solstice. Research is needed to determine why the body produces minimum and maximum melatonin levels with this lag. Perhaps a correlation could be found between this seasonal lag, the daily lag (peak melatonin concentrations occur eight hours after sunset) and new neuronal growth, sensitivity or activity.

The SCN contains neurons controlling antidiuretic hormone (ADH) release. ADH concentrations also occur with seasonal variations (9). The highest concentrations occur in the fall and the lowest concentrations occur in the spring. This is opposite in cycle to melatonin.

The SCN volume and ADH neuron concentrations are at a peak in fall and are lowest in summer (51). This reflects the seasonal variation in basal ADH concentrations. High concentrations of ADH inhibit the release of melatonin (118). Pinealectomy (a reduction in circulating melatonin concentrations) results in an increase in ADH concentrations. The feedback loop between melatonin and ADH may occur in the SCN, where ADH neurons and neuronal signals destined for the pineal gland are both processed with regard to retinal photoperiod input. ADH levels increase with age (9), as melatonin levels decrease along with a possible inhibition of ADH.

Growth hormone basal levels peak at puberty and then continuously decline with advanced age. This decrease in GH is due to a decrease in production and pituitary responsiveness to GH-releasing factor(132). Decreases in plasma GH cause a decrease in protein synthesis, reduction in kidney and liver function, as well as a loss of bone and immune system deterioration(112).

Growth hormone's response to GH-releasing hormone decreases with advanced age, as does prolactin's response to doperidone. Both GH and PRL secretions occur based on the 24 hour circadian cycle, depending on the sleep-wake cycle. GH and PRL are secreted in sleep, mirroring melatonin. GH gradually decreases in concentration with age, and prolactin's circadian periodicity is flattened out, losing its nocturnal peak. Melatonin supplements restore the peak secretion of prolactin at night, while dopamine decreases its secretion(17). Low levels of prolactin make the immune system less productive.

Growth hormone secretion mirrors the cyclic secretion seen in melatonin, with a nocturnal peak. A decrease in GH concentrations is seen in aged individuals. Although the

cyclic pattern of release is not compromised, the amplitude is decreased(124). GH normalization is an important goal for many aged individuals, as it may reduce the increased occurrence of sleep disturbances seen in the aged.(56)

Prolactin is secreted with a circadian rhythm in humans, usually with a nocturnal peak. Prolactin peak concentrations also occur during daytime sleep (131). This dual control of prolactin secretion, sleep and circadian rhythm results in jet lag symptoms seen in travelers of multiple time zones (29). The intrinsic circadian rhythm of prolactin secretion is not in sync with sleep. Melatonin supplementation at high concentrations can reset these intrinsic circadian rhythms of prolactin secretion to correspond with appropriate sleep hours. Inconsistent results show both increases and decreases in prolactin amplitudes in response to melatonin supplements in elderly individuals (129). The circannual rhythm of prolactin secretion appears to remain consistent with age, although this is usually reported predominantly in women. Consistent circannual rhythms of prolactin are difficult to detect in men.

Prolactin is mainly secreted and stored in the pituitary gland, as little prolactin is stored or secreted in the median eminence. Advanced age has no affect on prolactin storage in the pituitary, nor median eminence secretion. Age increases median eminence prolactin storage and decreases pituitary secretion. Melatonin supplements have no significant affect on cyclic nor acyclic mammals' pituitary prolactin storage. Melatonin increases pituitary prolactin secretion in cyclic mammals. Melatonin does not directly affect prolactin release, but inhibits the release of dopamine from the preoptic area of the hypothalamus. 17*B*-estradiol blocks melatonin's inhibition of dopamine. Dopamine's inhibitory action influences prolactin secretion. Therefore, indirectly, melatonin supplements in ageing individuals will raise the level of prolactin [Fig. 2]. However, in younger individuals, melatonin decreases prolactin production (106). Melatonin does not affect secretion nor storage of prolactin in the median eminence(2).

When luteinizing hormone-releasing hormone(LHRH) is supplemented, cyclic and acyclic rats showed significant increase in luteinizing hormone secretion, but only acyclic rats exhibited increased FSH secretion(2). This results from different hormonal control mechanisms for the two gonadotropins. It, also, appears that the control mechanisms may change in response to increased age, resulting in or as a result of decreased fertility or complete loss of fertility. Melatonin supplements neutralized the increased gonadotropin release stimulated by LHRH additions(2).

LH secreting cells, gonadotropes, in the pars tuberalis of mammals have a distribution corresponding to LHRH neuron terminals in the ME (10). LH inhibits its own release in a short-loop feedback by diffusing from the pars tuberalis into the ME and inhibiting LHRH release (81). This inhibition may be a direct action of LH on LHRH neuron terminals or an indirect action via other neuronal terminals.

Melatonin receptors in mammalian brains are found in the highest concentrations in the pars tuberalis (135). This leads to the conclusion that melatonin may inhibit LH release in the pars tuberalis of the pituitary gland. The endocrine secretory cells of the pars tuberalis undergo seasonal fluctuations in their histology reflecting reproductive cycles. These fluctuations may result from variations in pineal melatonin release promoting seasonal breeding.

Melatonin stimulates ME LHRH secretion. Melatonin acts on receptors found on gonadotrope cells in the pars tuberalis, inhibiting LH release (81). As LH inhibits ME LHRH secretion, melatonin indirectly increases ME LHRH secretion by blocking the LH short-loop feedback. Melatonin may inhibit LH release by inhibiting adenylate cyclase in gonadotropes, producing a reduction in cAMP. Significant melatonin receptor concentrations in the pars tuberalis are dependent on estrogen (62). When estrogen concentrations are low, such as after menopause; pituitary melatonin receptor concentrations decrease, and the inhibition of LH release is lost. Therefore, low estrogen

concentrations can lead to a decrease in ME LHRH release, although LH levels will remain high. Melatonin is intrical to the sensitivity of LHRH release [Fig. 3].

These age related changes in the hypothalamic-pituitary functions of the body affect reproductive hormones and, thus, reproductive viability. Deficient LHRH, or response and sensitivity to it, has been linked to decline in reproductive function(73). Age related changes in the anterior pituitary also produce a decline in reproductive function through gonadotropin secretion. Acyclic rats' anterior pituitary, when transplanted on the median eminence of cyclic rats, can not support cyclic secretion of gonadotropins(85). These cyclic rats show no loss of reproductive function when the anterior pituitary transplant is taken from another cyclic animal. These changes in the pituitary and hypothalamus coincide with the gradual decreased secretion of melatonin from the pineal gland, beginning in middle age.

Pituitaries of acyclic animals are still responsive to exogenous LHRH and melatonin. The altered state of the anterior pituitary results from changes in endocrine status, not simply chronological age. The acyclic, transplanted anterior pituitary glands that could not support normal estrous cycles had already been altered by the acyclic animals endocrine cycle. These glands were not responsive to regeneration when transplanted into a cyclic animal's hypothalamic-pituitary reproductive feed-back loop. There is no research available to determine the results of additional melatonin supplements to these cyclic-transplant animals with regard to acyclic anterior pituitary regeneration.

The biosynthetic mechanisms to produce gonadotropins and prolactin are unaffected by chronological age. The secretory response is altered with increasing age and relative to decreasing melatonin concentrations. Aged animals exhibit higher concentrations of circulating gonadotropins. The addition of melatonin supplements to the diets of ageing animals restores the concentrations of melatonin to levels exclusively

secreted by the pineal gland in youth. This lowers gonadotropin levels by inhibiting the response of the gonadotropin secretion to LHRH.

There is no change in prolactin, nor LH storage when melatonin is supplemented, although melatonin decreases the storage levels of FSH in young animals. This is the same result observed as animals age without melatonin supplements. Melatonin lowers gonadotropin secretion through LHRH, but it has no affect on LH storage, it acts the opposite, similar to age, as would be expected by lowering FSH storage in cyclic animals and raising FSH storage in acyclic animals. When storage is decreased, increased secretion would be expected. As storage is increased, a decrease in secretion would be expected. This counterintuitive fact would be an excellent area for further research to determine melatonin's biological interactions with gonadotropin receptors.

Chronological ageing decreases the hypothalamic regulation of LH(87). The body is capable of producing youthful levels of LH in advancing age, however this is prevented by the endocrine system. GnRH controls the release of LH from the pituitary. The expression of the GnRH gene is suppressed with increasing age. LH also becomes less responsive to GnRH with age. Increased levels of circulating melatonin increases the expression of the GnRH gene and the responsiveness of LH to GnRH(106). Melatonin supplements indirectly decrease the surge in LH that foreshadows menopause, thus delaying the onset of menopause.

A principle interest of melatonin research has been with regard to the female reproductive cycle. As melatonin is a tropic hormone, it has significant affects on reproductive cycles, as they are based on multiple hormones. The pituitary-hypothalamic axis is the center of regulation for each cycle.

In ageing females, melatonin's effects are complex. If melatonin is supplemented in premenopausal individuals, it can delay menopause.

Women are delaying having children until later in their lives. As reproductive fertility is almost completely lost ten years before menopause, women must race their biological clocks. They gamble with increased risks of miscarriages or Down's syndrome.(76) With recent changes in societal pressures, women must balance these risks of abnormality or infertility with establishing a career before having children. Ten percent of women start menopause before they are forty-five.(76) These ten percent could completely lose their fertility by the age of thirty-five. These pressures are evident in the increased reliance on fertility drugs. Meredith contrasted three methods to delay menopause.(76) Caloric restriction is the first. As this restriction must be chronic, it is obviously unfeasible. It is interesting to note that caloric restrictions extend human lifespans and the intestine is one area that produces melatonin in the human body. Research should explore the link between caloric restrictions and higher levels of melatonin in greater depth. Melatonin may also regulate the patterns we develop regarding hunger and meal times.

The second method to delay menopause is long-term progesterone supplementation(61). The progesterone is slowly administered from silastic implants. Progesterone supplementation has undesirable side affects, including bleeding irregularities (117). Progesterone supplementation and caloric restrictions cause the nervous system, hypothalamic-pituitary axis, to decrease the amount of LH secreted with age(61). Both methods also reduce the age related loss of primordial follicle cells. With more follicle cells, inhibin concentrations remain high enough to inhibit FSH secretion.

The final method to delay menopause is through melatonin supplements. Nighttime supplementation of melatonin had no effect on the number of primordial follicle cells(76). Melatonin is a tropic hormone and, therefore, is the most natural and noninvasive way to delay menopause. Melatonin supplements replace the reduced levels of melatonin secreted by the pineal gland with age. This allows pituitary secretion of gonadotropic hormones

similar to levels found after puberty and before menopause. There are few side effects, although Pierpaoli found an increased occurrence of ovarian tumors exclusively in one species of mice, C3H/He.(90) Nighttime supplementation of melatonin delayed menopause by regulating the length of estrous cycles through gonadotropins late in reproductive life.(76) Twenty-four hour supplementation did not affect the reproductive lifespan (76).

After menopause, melatonin exhibits different reactions on the hypothalamus-pituitary axis. Post menopausal ageing decreases the pituitary's gonadotropic regulation. FSH and LH levels remain much higher than in youth. LH becomes less responsive to GnRH with increasing age after menopause.

Estradiol and progesterone levels decrease in women after menopause. LH and FSH plasma levels are elevated in elderly women.

Melatonin supplements have been shown to increase the LHRH mRNA levels significantly in older individuals(101). A decrease in either LHRH or the pituitary's response to LHRH begins the decline in reproductive function and a LH surge.

The inhibitory affect of melatonin on the secretion of pituitary hormones is dependent on the endocrine status and age of the animal(100).

Prepubertal males' androgens exhibit a negative feed-back on hypothalamus activity, specifically gonadotropin secretion. Testosterone propionate inhibits LH secretion. The sensitivity of this feed-back loop decreases after puberty, which it initiates(72). Plasma LH concentrations remain similar after puberty. With the decreased sensitivity to androgens, higher concentrations of testosterone are required to maintain the same levels of LH. Testosterone exhibits a positive feed-back effect on prolactin in prepubertal males, but not in adult males(31).

As menopause is not an event in the male reproductive life, research regarding melatonin's effects on the neuroendocrine-reproductive axis are tailored towards puberty, in order to diagnose and treat problems that develop at this age. This research focuses on

the development of sexual organs, starting with the fetal endocrine system. The negative feed-back response of LH to testosterone begins in the fetus. This feed-back becomes less sensitive, initiating puberty by requiring higher concentrations of plasma testosterone to maintain the same plasma concentrations to prepubertal LH levels, and coinciding with an increase in GnRH receptors in the pituitary gland(31). As testosterone serum concentrations rise, they inhibit the hypothalamic release of GnRH(31). Higher concentrations of GnRH receptors are found in the pituitary, with puberty, in order to maintain proper LH plasma concentrations(31). When melatonin is administered to prepubertal males, the negative feed-back testosterone propionate exerts on LH is inhibited(31).

Melatonin reduces the negative feed-back affects produced by testosterone propionate on LH, but lowers the basal levels of LH in prepubertal males. Melatonin inhibits GnRH release, hypothalamic-pituitary axis sensitivity to gonadotropins and the reproductive cycle, delaying puberty.

After puberty, which results in lower plasma concentrations of melatonin and higher plasma concentrations of testosterone, additional melatonin supplements raise the basal concentrations of plasma LH. This is the same result as seen in females after puberty. Melatonin supplements continue to inhibit the affects of testosterone propionate on LH secretion, as found before puberty.

Melatonin lowers the plasma LH concentrations before puberty even though it also reduces the inhibition of testosterone propionate on LH. Melatonin lowers the plasma levels of LH before puberty by acting on the hypothalamus, not the pituitary. This is accomplished through the decrease of GnRH release from the hypothalamus. After puberty, there is less significance regarding melatonin's reduction of hypothalamic GnRH release because the GnRH receptor concentration increases in the anterior pituitary.

Pituitary FSH secretion is inhibited by testosterone to a lesser degree than LH, as gonadotropin release is regulated by different mechanisms(40). FSH secretion is not only controlled by testosterone, but also by inhibin B(128). Before puberty, melatonin lowers basal plasma FSH concentrations. Although, unlike LH, melatonin has little affect on the negative feed-back exerted by testosterone propionate on FSH prior to and after puberty(31).

The reduced basal plasma concentrations of FSH due to melatonin do not change with the addition of testosterone propionate(31). These basal concentrations are lower than the inhibitory effect testosterone propionate produces without melatonin.

Melatonin lowers the basal plasma concentrations of both gonadotropins prior to puberty In this manner, melatonin supplements can delay the onset of puberty. Basal prolactin levels are at the highest plasma concentrations at puberty. Before and after puberty, testosterone exhibits a stimulatory affect on prolactin. During puberty, testosterone's stimulatory affect on prolactin is most pronounced. Melatonin reduces the basal plasma concentrations of prolactin before puberty and during puberty. Melatonin reduces testosterone's stimulation of prolactin secretion(31). Melatonin's reduction of testosterone's response on prolactin reduces in potency with age, from birth until death. Testosterone's stimulation of prolactin is at its maximum during puberty. Melatonin greatly reduces this stimulation of prolactin secretion at puberty.

Elderly men exhibit low plasma testosterone concentrations in the morning(132). In the afternoon, the plasma testosterone levels of elderly men are similar to those of younger men. As melatonin production decreases with age and is at its highest levels in the early morning, melatonin may not only amplify the tropic affects of testosterone, but also contribute, with short duration, to its production or secretion.

The rhythm and concentration of melatonin secretion was disturbed in hypogonadal male patients (66). Testosterone replacement normalized pineal function

Melatonin affects gonadotropin and gonadal steroid secretion. In addition to melatonin's influence on the pituitary gland, human granulosa cells (138) and prostate cells (39) also have melatonin receptors. Recent studies have suggested that melatonin secretion may be influenced by gonadotropins and gonadal steroids. LH, FSH, estrogen and androgen receptors are present in the pineal gland (65) As the pineal gland calcifies, melatonin secretion decreases, yet gonadotropin and gonadal steroid receptors persist in the pineal gland with age. Gonadotropin receptors vary in concentration with season, expressing a ten- fold increase in winter. Seasonal changes in photoperiod duration produce these changes. Daily light-dark cycles only produce changes in FSH pineal receptor concetrations, with a higher concentration at night. Plasma concentrations of gonadotropins do not change between seasons. The seasonal concentration changes in pineal gonadotropin receptors in the pineal is similar to the winter increase in melatonin secretion, storage and SCN vasopressin cell concentrations (65). Gonadal steroids appear to only influence melatonin release at night (113). Although, this time- related effect does not change gonadal steroid pineal receptor concentrations. It may be the combination of gonadal steroid receptor binding and the influence gonadal steroids have on gonadotropin secretion on the hypothalamus- pituitary- gonadal axis. Gonadal steroids and gonadotropins effect the peptide and indole secretions in the pineal gland (44). These peptides and indoles may also have an influence on melatonin synthesis and secretion.

TACHYKININS

Tachykinins are bioactive peptides that stimulate smooth muscle. Although they have widespread localizations, they are predominantly concentrated in the respiratory and gastrointestinal tracts. Tachykinins are vasodilators (96) and influence the secretion of endocrine glands. This regulation of blood supply modifies tachykinin concentrations in a feedback loop with reproductive hormones. Tachykinin's paracrine activity acts on the hypothalamus, anterior pituitary, gonads and pineal gland, modulating the hypothalamo-pituitary-gonadal axis (23).

Four precursor proteins produce the tachykinins. These proteins are the products of two genes, preprotachykinin A and preprotachykinin B (23). Preprotachykinin A encodes for substance P (SP), neuropeptide K (NPK), neurokinin A (NKA), and neuropeptide *y* (NPG). Preprotachykinin B encodes for neurokinin B. Two of the four precursor proteins contain the complete sequence of SP and NKA, formation of each depending on RNA splicing and posttranslational processing (23). As a result, SP and NKA are generally colocalized.

Tachykinins have three subtypes of receptors: SP targets NK-1 receptors, NKB targets NK-3 receptors and NKA, NPK and NPG target NK-2 receptors (22,27). Tachykinins interact with all three receptor types, as they display poor selectivity (126). NK-2 receptors are generally located in the peripheral nervous system (101), while NK 1 and NK-3 receptors are distributed in the central nervous system (26).

Successful reproduction depends on an interrelationship between the hypothalamus and pituitary gland, and an interaction of the pituitary gland and gonads via gonadotropic hormones (23). Reproductive hormones and tachykinins modulate the regulation of the

hypothalamus, the anterior pituitary, and the gonads (the three levels of the reproductive system).

The hypothalamus has an abundance of SP, NKA and NKB neurons; including the preoptic area, ventromedial nucleus, infundibular nucleus and arcuate nucleus. The preoptic nucleus influences the sexual behavior of male rats, as male rats have double the tachykinin concentrations of female rats (23). Large bundles of tachykinin-positive nerve fibers are found in the anterior suprachiasmatic nucleus, connecting it with tachykinin-containing retinal ganglia cells (93). This is also the first step in stimulating the pineal production of melatonin.

The ventromedial nucleus of female rats is responsible for sexual receptivity. The concentration of tachykinins in the ventromedial nucleus of male rats remains constant with castration. Female rats' tachykinin concentrations increase with estrogen exposure and decrease with ovariectomy (100). Nerves containing tachykinin transcripts and estrogen receptor mRNA, in the infundibular nucleus, hypertrophy after menopause (23). These nerves may help regulate estrogen and gonadotroph negative feedback. The arcuate nucleus, a center of neuroendocrine control, receives a large tachykinergic innervation from the preoptic area (98). The arcuate nucleus also has the highest tachykinin concentration of the hypothalamus (67). The SCN is connected to both the retina and arcuate nucleus via dense tachykinergic nerve fibers.

The tachykinins modulate the secretion of hypothalamic hormones by stimulating neurosecretory terminals, which release the hormones into the portal vessels (19). Vasopressin and oxytocin release may be modulated by tachykinin receptors in the supraoptic and paraventricular nuclei. Tachykinergic neurons and magnocellular neurons in the supraoptic and paraventricular nuclei have synaptic contacts (19).

Tachykinins modify gonadotropin secretion via hypothalamic GnRH release. Thus, gonadal steroids may modulate tachykinin concentrations in the hypothalamus via their

negative feedback loop with GnRH (19). Orchidectomy in rats decreased the median eminence, paraventricular nucleus, arcuate nucleus (75) and hypothalamic NKA concentrations (35). Testosterone, dihydrotestosterone and extradiol treatments reversed the NKA declines seen in each of the previous experiments, returning NKA concentrations to the level of controls, or higher, in the central nervous system. In addition to the regulation of tachykinins that testosterone and estradiol exhibit, GH may also alter tachykinin concentrations (42).

The anterior pituitary gland contains a lower concentration of tachykinins than the hypothalamus (46). Male rats' anterior pituitary tachykinin concentrations are higher than female rats' concentrations (31). Although this observation was based on a mean measurement, tachykinin concentrations fluctuate in the anterior pituitary of female rats during the estrous cycle (46). Tachykinin concentrations in the anterior pituitary are highest when estradiol serum levels are lowest. Estrus and tachykinin concentrations are lowest when estradiol serum levels are highest, proestrus. The estradiol suppression of tachykinins in the anterior pituitary is the reverse of the estradiol influence on the hypothalamus. Ovariectomy resulted in a rise in anterior pituitary tachykinin concentration, and estradiol treatment reversed the increase (46). Male rats, also, displayed an anterior pituitary tachykinin concentration increase with orchidectomy, which was blocked with testosterone replacement treatments (38). Additionally, thyroid hormones inhibit tachykinin concentrations in the anterior pituitary gland (72).

Anterior pituitary glands have lower concentrations of tachykinins than hypothalamus glands, but the tachykinin levels are more sensitive to environmental photoperiods. The Siberian hamster, as a species, is extremely sensitive to environmental photoperiods, and possesses a relatively higher concentration of tachykinins in its anterior pituitary, with regard to other rodent species (30). Shortened and lengthened photoperiods increased tachykinin concentrations in the anterior pituitary of the Siberian hamster. The

animals exposed to the shorter photoperiods had the largest increase in tachykinin concentrations. Gonadal regression occurred in animals exposed to altered photoperiods. In females, the decrease in gonadal hormones resulted in the increase in anterior pituitary tachykinin concentrations. In males, decreased thyroid hormones resulted in the increase of anterior pituitary tachykinin concentrations (40). The thyroid regression resulting from the altered photoperiod would also contribute to the tachykinin increase in females, as this increase is sufficiently strong to offset the loss of tachykinin stimulation from testosterone in gonadally regressed animals.

Substance P, NKA and NPK tachykinins are found in the pineal gland (32,55). Tachykinins in the pineal gland are inhibited by testosterone. Castration produces an increase in pineal tachykinin concentrations, and these concentrations return to normal with testosterone replacement therapy (32). In female rats, ovariectomy resulted in a decrease in pineal tachykinin concentrations. Superior cervical ganglionectomy, the main noradrenergic pineal innervation, resulted in an increase in pineal tachykinin concentrations. Although this innervation, the final step relaying circadian rhythm to the pineal, produces an inhibitory effect on pineal tachykinin concentrations, and pineal tachykinin concentrations decrease with dark photoperiods in female rats, no significant circadian rhythm of pineal tachykinin concentrations was found (32). The decline in pineal tachykinin concentrations in female rats during dark photoperiods may be an indirect response to the influence gonadal hormones exhibit on tachykinin concentrations.

Tachykinins increase the prolactin secretion, particularly during proestrus surges, in the presence of a significant estrogen concentration (23). When an antiserum to NKA was injected during diestrus II, proestrus prolactin secretion was significantly decreased (94). Hemipituitaries incubated with an anti-NKA serum had reduced basal and NKA stimulated prolactin secretion in intact rats (95). The same results could not be reproduced with ovariectomized rats, lacking estrogen. Antiserum to NKA also reacts with NPK and

NPG. In addition, antiserum to NKA (96) and anti-SP serum (24) reduced the prolactin surge resulting from suckling. Henriksen found tachykinins to stimulate prolactin secretion in anterior pituitary cells (49). The tachykinin stimulation of prolactin release may act directly on the anterior pituitary, or it may be directed at blood-brain barrier deficient hypothalamic neurons. Tachykinins may alter the release of neurotransmitters from these neurons, affecting prolactin secretion in the anterior pituitary (23). Additional research is needed to determine if tachykinins affect prolactin releasing hormone in the hypothalamus.

NKA may stimulate prolactin secretion by activating phosphoinositide metabolism (69). NKA activates the receptor-mediated hydrolysis of phosphoinositides in the anterior pituitary, which stimulates prolactin and LH secretion (74). NKA intracerebroventricular injections increased proopiomelanocortin (POMC) mRNA expression 48% in the arcuate nucleus (69). Opioids stimulate prolactin secretion and inhibit LH secretion (23). NKA activation of POMC neurons may influence an interrelationship between tachykinins, prolactin and gonadotropin release.

NKA activation of the opioid system may decrease LH secretion by inhibiting GnRH release at the hypothalamic level (23). The NKA inhibition of LH secretion may also be a result of the tachykinins suppression of neurotransmitters at the hypothalamic level, or a combination of the two mechanisms (23). When the third ventricle of ovariectomized rats is injected with NKA, plasma LH concentrations decrease (23). When injecting an antiserum to NKA with cross-reacting activity for NPK and NPG into the third ventricle, serum LH concentrations increase in ovariectomized rats. Without the cross-reacting activity for multiple tachykinins, antiserum to NKA will not elicit a change in plasma LH concentrations (94). This appears contradictory to experiments resulting in decreased LH plasma concentrations when NKA is injected into the third ventricle. Intraventricular injection of NPK produced a significant reduction in plasma LH, and NPG intraventricular injections reduced plasma LH concentrations to a lesser extent. The LH

surge produced by progesterone could also be inhibited by NPK. The three tachykinins, working together, produce a strong physiological inhibition of plasma LH secretion. Perhaps the cross-reactivity is important because NKA, NPG and NPK share NK-2 receptors. A general NK-2 receptor agonist was shown to inhibit basal hypothalamic GnRH secretion (57).

Male rats intraventricularly injected with NKA, NPG or NPK had significant increases in serum LH (57). When orchidectomized, the tachykinin intraventricular injections suppressed LH serum concentrations. As in females, NPK was the most potent. NPK and NPG stimulate LH release at the pituitary level of intact male rats. An NK-2 antagonist stimulated LH, but not FSH, secretion from the pituitary (20), but inhibited the LH and FSH response to GnRH (23). NPK was unable to inhibit LH release when injected with NMDA. Tachykinin inhibition of LH release appears to be related to a suppression of neurotransmitters.

Tachykinins generally suppress LH release at the hypothalamic level and stimulate release at the pituitary level. The actions of tachykinins are affected by gonadal hormones, which can negate the effects of tachykinins. Tachykinins can not stimulate LH release at the pituitary level without the presence of testosterone (57). Tachykinins exert an effect on the hypothalamus-pituitary axis at multiple points. They react with GnRH neuron cell bodies in the preoptic area, median eminence nerve terminals and neurotransmitter suppression (23). These multiple points of influence, combined with gonadal hormone modification of tachykinin effects, create an intricate regulation of the endocrine system with multiple feedback loops.

Tachykinins help regulate hormone secretion in gonads. SP is found in Leydig cells (19) and tunica albuginea nerve fibers (124). The testis have also exhibited the ability to synthesize SP, NKA, NPK and NPG (23). SP inhibits testosterone release by Leydig cells (5). NPG and NKA stimulate estradiol secretion from Sertoli cells. This increase in

estradiol results from an increase in testosterone secretion, enzymatically being synthesized into estradiol (23). If tachykinins stimulate Sertoli cell secretions of testosterone, they may stimulate the Sertoli secretions of other factors, influencing the hormone secretion of Leydig cells. The tachykinin inhibition of testosterone release from Leydig cells occurs in both long and short photoperiods (5), although SP reduced the Leydig cell LH receptor concentrations during short photoperiods (58).

Ovarian SP concentrations dramatically rise after puberty (84). SP effects on ovarian hormone release differs before and after puberty. In 15 day old hamsters, SP stimulated estradiol release, but not progesterone release. Adult hamsters' ovaries incubated with SP had stimulated progesterone release, and inhibited estradiol release (5).

Melatonin is a tropic hormone, influencing reproduction with its affects on gonadotropic hormones, estrogen and testosterone feedback loops, prolactin and GH. Tachykinins also help maintain the hormonal balance needed for reproduction. Both melatonin and tachykinins act on the hypothalamo-pituitary-gonadal axis to achieve this hormonal control. Tachykinins' primary influence on the hypothalamo-pituitary-gonadal axis is as vasoactive peptides. They can alter the blood flow to neuroendocrine structures, including the pineal gland. Tachykinins also display paracrine and autocrine activity. This allows for specific neuronal effects, such as stimulating GnRH or prolactin secretion (23). Tachykinins are essential to anterior pituitary and possibly hypothalamic hormone release. Tachykinins stimulate LH and prolactin secretion from the anterior pituitary gland. Gonadal steroids affect the tachykinin concentrations in the pineal gland (23). Perhaps tachykinins have a more direct mode of regulation on melatonin secretion, production and feedback loops than vasoregulation.

The hypothalamus, median eminence, pituitary gland and gonads are coordinated through complex nerve and hormone feedback loops. Tachykinins and melatonin affect similar hormones, influencing their storage and secretion. Melatonin and tachykinins

innervation is also similar. The SCN receives innervation from the retina that, once processed, will eventually reach the pineal gland. The anterior SCN also has large bundles of tachykinin-positive nerve fibers, connecting it to the retina. The preoptic area projects a tachykinin innervation to the arcuate nucleus. The arcuate nucleus, the highest tachykinin concentration in the hypothalamus, is connected to the SCN. It may be possible that neuroendocrine information is combined with photoperiod signals, processed in the arcuate nucleus and sent to the pineal gland for appropriate release of melatonin. Other nuclei may also be connected to the SCN for this purpose. The infundibular nucleus nerves containing tachykinins, hypertrophy after menopause. Additional research exploring the possible coordination of tachykinin and photoperiod influences on infundibular nucleus actions affecting melatonin regulation is needed.

IMMUNE SYSTEM

If age is a result of the deterioration of the immune system, or vice versa, the thymus must be viewed as an important factor. The thymus is replaced by fatty tissue with age, until it hardly exists as a functional gland. Regelson has shown melatonin to increase the weight of the thymus, restore the production of T cells from the thymus and restore the antigen response of T cells (92). These results appear amazing, but Regelson produced more incredible results. He restored a thymus gland, ravaged by time, to its youthful, functional ability by transplanting an active pineal gland on to this thymus gland.

Preventing the nighttime surge of melatonin caused a reduction of antibody production in mice. Restoring the melatonin surge in the same mice resulted in a return of antibody levels to normal (122). Melatonin production decreases with age. High fat diets reduce T cell function, and insufficient vitamin C leads to lower T cell numbers. Smoking tobacco decreases the available vitamin C to the body. Older smokers with fatty diets frequently have many medical debilitations. Certainly, a contribution to these ailments is the accelerated decline in their immune systems. Increasing health in the elderly should include a healthy diet, not smoking and melatonin supplements to maintain a strong immune system.

Sleep deprivation produces a decline in T cell function (92). The immune system deterioration directly reflects ageing. The thymus shrinks and T cells become less effective as an individual ages. Particularly, suppresser T cells become almost ineffective. This allows antibodies to destroy valuable, non-antigen cells in the body.

Melatonin increases the antibody response when supplemented after initial exposure to antigens. Regelson provoked an immune response in mice by exposing their

immune system to sheep blood, after an initial exposure to produce antibodies (92). Their immune response was increased when melatonin was supplemented for one week after the initial exposure. Regelson raises the question, would melatonin supplements increase the effectiveness of childhood vaccinations? Although possible, this may not be necessary, as melatonin levels are highest prior to puberty.

Many cells produced by human lymphoid organs, i.e., thymus, spleen and blood, have melatonin specific membrane bound receptors with an affinity between 0.1-1 nM Kd (13). Melatonin, at its physiological concentration, appears to partially regulate the immune system by directly regulating immunocompetent cells (136). Melatonin receptors have also been found on the cell membranes of the retina, SCN, pars tuberalis, thalamus, subiculum and area postrema (137). Melatonin is an important common signal molecule with receptors on both neuroendocrine and immune cells. This simultaneous influence on both systems allows melatonin to synchronize the endocrine and immune system, helping them to work in concert.

Human blood lymphocytes have two different melatonin specific binding sites (16). One receptor is high affinity, while the other is low affinity. The high affinity receptor has a 5-50 times lower affinity for melatonin than the receptors found in the ME, SCN, area postrema, and Harderian gland (16). Although melatonin exhibits a relatively weak affinity for lymphocytes, high affinity lymphocyte receptors have a Kd value slightly higher than serum melatonin concentrations at their physiological peak, at night (16). Therefore, lymphocytes may only be influenced by melatonin at night. Lymphocytes appear to be the only leukocyte with the high affinity melatonin receptor. The receptors found in granulocyte cells are similar in affinity to the low affinity receptors of lymphocytes. For example, neurtophils have a melatonin receptor with an affinity in the *u*M range (16). Often, melatonin serum concentrations are too low to bind to the high affinity lymphocyte receptor. With 1000 times lower affinity, it is unlikely that the granulocytes are directly

influenced by physiological concentrations of melatonin. This does, however, allow the option of influencing granulocytes through prescription doses of melatonin. Further research to discover the purpose of these melatonin receptors on granulocytes and what effect high doses of melatonin have on the high affinity recetors would be valuable.

Melatonin binding sites have been found in spleenocytes (102) and thymocytes (138). These binding sites are considered high affinity with a Kd of 0.34 and 1.72 nM, respectively (16). The 1.72 nM Kd affinity of melatonin receptors in the thymus is relatively strong. Melatonin, at physiological serum concentrations, enhances the immune system by inhibiting the influence of corticosterone (139). Although, this result was only exhibited when the animal was first infected with T-dependent antigens (16). This decisively proved that melatonin's influence on the thymus existed. Melatonin was later shown to increase the affinity and decrease the concentration of adrenal steroid receptors in the thymus (16). This solidified melatonin's role as an inhibitor of immunosuppressive agents, such as steroid hormones. The thymus receptors for steroid hormones are a site of interaction between the immune and endocrine system. Melatonin and steroid hormones interact on thymocytes and influence the extent and duration of the immune response (86).

The number and affinity of melatonin receptors in the thymus changes with age (15). The pineal gland does not fully develop until the second week after birth (42). This results in a low serum concentration of melatonin. The melatonin receptors on thymocytes increase their binding capacity via an increase in receptor numbers, not affinity, in order to compensate. Melatonin binding to thymocyte receptors is higher in newborns than adults. Pinealectomy in neonatals reduces thymus size and immune function (16). Melatonin is intricately involved in the maturation and function of the thymus and immune system.

The development of the pineal gland during the first two weeks after birth may be affected by an infant's first exposure to direct light. As the pineal develops, melatonin serum concentrations increase. This increase reduces the negative feedback which was

increasing the concentration of melatonin receptors on the thymus (16). The loss of receptor concentration is seen as the thymus and pineal gland develop.

When animals are exposed to constant light, an increase is seen in melatonin's binding to spleenocytes (102). This increase in receptor concentration, again not affinity, may allow light to influence melatonin's regulaton of lymphoid cells (16). As light levels increase, melatonin concentrations decrease and receptor binding increases.

Recently, the hypothesis for the signal transduction pathway for melatonin in human lymphocytes and its affect on cAMP and cGMP production was solidified at The University of Seville in Spain (16). Melatonin was found to be unable to activate cAMP production. Vasoactive intestinal peptide (VIP) can activate cAMP production in human lymphocytes. When melatonin, at physiological concentrations, was combined with VIP, an increase in cAMP production was observed exceeding the production of VIP alone. This effect was not seen with any other peptide in the secretion-VIP group. Both VIP and melatonin may be needed to regulate cAMP production in human lymphocytes, thus influencing the immune system, as the effect is seen at physiological concentrations of both melatonin and VIP. A similar result was observed on spleenocytes when melatonin was combined with forskolin (102). Alternatively, when pharmacological doses of melatonin were administered to spleenocytes, cAMP production was inhibited.

Melatonin, alone, directly increases the cGMP production in human lymphocytes up to 10 *u*M (136). GTP inhibited the binding of melatonin to its membrane receptors. ATP, CTP and UTP did not affect melatonin binding (16). This affirmed the conclusion that melatonin receptors are coupled to G-proteins in human lymphocytes.

Thyrotropin (TSH) is secreted with a circadian rhythm and peaks nocturnally. This cyclic rhythm is lost in aged individuals, in whom TSH secretion follows an increasingly linear pattern. T4 is not secreted with a circadian rhythm, although its secretion and synthesis is controlled by TSH. T4 secretion produces a negative feedback control on

TSH. This interdependence would infer a circadian rhythm for T4 similar to TSH secretion, but these secretion patterns are complicated by thyrotropin-releasing hormone (TRH). TRH influences the hypothalamic release of TSH.

CANCER

Patients with unoperated primary prostate cancer (malignant) express different hormonal concentrations. TSH secretion is depressed with a further depression at 19:00 hours (13). The circadian rhythm of secretion is also lost, as in patients with benign prostate hyperplasia. The T4 plasma concentrations are slightly higher in men with primary prostate cancer than in men with benign prostate hyperplasia. This T4 elevation inhibits TSH production as a result of the increased negative feedback on TSH (13)

Many elderly men develop benign prostate hyperplasia, and the risk of developing malignant cancer increases with age. A current diagnostic tool to detect benign prostatic hyperplasia or prostatitis is the prostate-specific antigen test (PSA). A high PSA result may lead to a biopsy and eventually an ambiguous Gleason score. Research is needed to determine if melatonin would be a beneficial treatment to inhibit a slow growing tumor. With the uncertainty regarding diagnosis of prostate cancer in men, melatonin supplementation may be a more beneficial, conservative treatment, alleviating the drawbacks of current aggressive treatments.

Melatonin plasma concentrations are lower in the elderly, but aged men with primary prostate cancer express even further depressions in melatonin secretion with an almost total loss of circadian rhythm (13). As the tumor increased in mass, the melatonin plasma concentrations continued to decrease. Melatonin secretion declines with age, but the large decreases of melatonin seen in cancer patients is not related to age. Patients with benign tumors expressed higher melatonin concentrations than patients of the same age with malignant tumors. The stage of a malignant tumor directly influences the depression of melatonin production and secretion (13). Melatonin concentrations in patients with

benign lesions are influenced by the individuals age, independent of the tumor. This conclusion holds true for breast cancer, as well as prostate cancer. Next to lung cancer, these are the most abundant cancers. It is logical to conclude that malignant tumors cause disruptions in hormonal concentrations Many cancers are hormone dependent. Breast cancer growth is estrogen dependent. As a result, estrogen levels are elevated in patients with breast cancer.

As melatonin levels are significantly lower in cancer patients, malignant tumors directly influence pineal secretion or production of melatonin (13). Melatonin, in turn, affects the concentrations of other hormones via its control of hypothalamic hormonal release. The decreased TSH levels may be a result of decreased melatonin concentrations producing a decrease in TRH stimulation. This leaves the increased T4 plasma concentrations in cancer patients unexplained, although high T4 levels further depress TSH secretion.

As a malignant tumor increases in size, melatonin production is increasingly inhibited. This negative feedback could account for the progression of malignant tumors. Melatonin supplements boost the immune system by restoring T cells to their most effective levels (92). With lower melatonin levels, a weakened immune system would be less effective against malignant tumors.

Generally, the suppresser T cells are less effective at recognizing self from non-self tissue. This causes many autoimmune diseases associated with age. The GH decrease seen with age causes both a decrease in the immune system and a change in protein synthesis. This slight change in the way a protein is composed may be why T cells are less effective at recognizing which tissues are self and which are foreign (92).

Melatonin is actively being researched as a drug to slow ageing and return hormone balances to youthful standards. In this respect, melatonin may be able to do more than help treat disease. Melatonin may be a way to prevent disease. The research strongly

suggests melatonin's value may primarily lie in preventing autoimmune diseases associated with age, for example, rheumatoid arthritis (92). Although this research is promising, due to the relatively long life of human beings, it may be many decades before these results are confirmed.

Melatonin also has benefits treating diseases. Cancer has been one of the deadliest diseases in human history. Many resources have been allotted to research treatment of cancer. As cancer is not a foreign invader of the body, it is difficult to prevent or treat. Cancer may be a natural consequence of age. The risk of cancer increases proportionally with age. With lower plasma melatonin levels and a weaker immune system, cancer may find less resistance to its establishment and growth in the body. Melatonin supplements and a strong immune system may lower the incidence of cancer in older people, but cancer will still occur. Melatonin may also help boost the effectiveness of current cancer treatments.

Cancer acts on the biosynthetic production of melatonin. This was deduced at the University Women's Hospital in Tubingen Germany by eliminating the following possibilities. First, malignant tumors did not accelerate the hydroxylation of melatonin in the liver. Detection of melatonin's primary metabolite (6-sulfatoxymelatonin) in urine samples of patients with malignant tumors was found in relative concentrations to melatonin as seen in patients with benign tumors (13). The maintenance of circadian profiles also rules out the possibility of a competitive interaction by an introduced enzyme. This knowledge is useful in detecting current pineal activity in cancer patients (13).

Second, N-acetylserotonin levels are much higher in cancer patients than age-matched controls, while serotonin levels remained similar. This increase is not a result of reconversion from melatonin, as the levels of N-acetylserotonin are close to one hundred fold higher than the levels of melatonin (13). This leads to the conclusion that N-acetylserotonin levels are prevented from being converted into melatonin by tumors.

Hydroxyindole-o-methyl transferase converts N-acetylserotonin into melatonin. Malignant tumors may produce responses in the immune system that interfere with the activity or availability of hydroxyindole-o-methyl transferase. More research is needed in this area.

Melatonin inhibits the growth of hormone dependent human breast tumor cells, for example estrogen- responsive MCF-7 cell line. Melatonin's suppression of MCF-7 growth is a combination of stimulating growth- inhibitory factors and down- regulating the expression of estrogen receptors (78).

Estrogen and progesterone receptors are found on MCF-7 cells. MCF-7 cells are estrogen dependent, requiring estrogen for regulating their growth via RNA transcription and protein expression (33). Estrogen is autoregulatory, altering the concentrations of estrogen receptors in MCF-7 cells for optimal growth rates. Melatonin's influence over MCF-7 growth is not with regard to estrogen, but the estrogen- response pathway. Only human breast cancer cell lines with estrogen receptors are susceptible to the antimitogenic effects of melatonin (50). Estrogen receptor protein production and estrogen binding affinity to estrogen receptors are decreased when physiological concentrations of night time melatonin are present. The rate of transcription of the estrogen receptor gene is decreased as a response to melatonin. Without estrogen receptors, MCF-7 cells could not regulate the key growth- regulatory factors, transforming growth factor alpha and beta (TGF alpha and TGF*B*) (78). Melatonin reduces the estrogen receptor concentration, stopping MCF-7 cell proliferation without regulation of growth factors or oncogene expression.

Transforming growth factor- *B*, an inhibitory growth factor, production is decreased by estrogen (60). Melatonin decreases the production of estrogen receptors and reduces estrogen's affinity for its receptors. This action indirectly results in a TGF*B* increase and an inhibition of MCF-7 cell proliferation. This supported melatonin's inhibition of MCF-7 cell growth via the estrogen- response pathway. Melatonin's increase

in TGF*B* expression is similar to the mechanism of tamoxifen in inhibiting estrogen receptor positive breast cancer cell proliferation (60).

The early proto- oncogene *c-fos* transcripts are increased in number as a result of estrogen. *C-fos* increases MCF-7 proliferation and differentation. Molis found melatonin to initially increase *c-fos*. After this intial increase, melatonin significantly inhibits *c-fos* production by almost 80% (78). This reduction in *c-fos* would result in a reduced occurance of normal cells differentiating into malignant cells.

PgR and pancreatic spasmolytic polypeptide (pS2) are MCF-7 intracellular proteins and easily referenced markers of estrogen receptor activation (78). PgR and pS2 gene expression are enhanced by estrogen at the mRNA level. Melatonin inhibits the mRNA expression of PgR and estrogen receptors. Although the estrogen- response pathway regulates PgR production, melatonin does not inhibit PgR production via the estrogen- response pathway, as mRNA levels for PgR and estrogen receptor proteins decrease simultaneously. Melatonin inhibits production of estrogen receptor proteins and PgR individually at the transcriptional level.

Melatonin's influence over the transcription of pS2 is also independent of the inhibition of estrogen receptor protein transcripion, as melatonin stimulates pS2 transcription. This difference in melatonin's regulation of MCF-7 intracellular, estrogen stimulated proteins suggests multiple mechanisms for inhibition of estrogen dependent cell proliferation, in addition to a reduction in estrogen receptor concetration (78).

The proto- oncogene *c-myc*, contributing to cellular proliferation and diferentiation, is stimulated by both estrogen and melatonin (78). Melatonin may regulate *c-myc* and *c-fos* with different mechanisms. Increased *c-myc* mRNA production, in response to melatonin, seemingly contradicts melatonin's inhibition of MCF-7 proliferation. Although, *c-myc* is a complex proto- oncogene, associated with tumor cell apoptosis at high concentrations (8). As *c-fos* and *c-myc* production are both stimulated

by estrogen, inhibition of both proto- oncogenes would be expected if melatonin reduced estrogen receptor concentrations. *C-myc* and *c-fos* mRNA expression both initially increase when exposed to melatonin. *C-myc* concentrations remain elevated, and *c-fos* concentrations steadily decrease after the initial elevation. Not only are different regulatory mechanisms suspected, the mechanisms may be initiated in time dependent stages (78).

Melatonin's inhibition of PgR and *c-fos* production and increase in *c-myc*, pS2 and TGF*B* production may be a signal transduction effect resulting in an early stage inhibition of estrogen dependent cancer cell proliferation (78). The signal transduction may be triggered by a cell surface melatonin receptor. After six hours, melatonin begins to reduce the estrogen receptor concetrations, resulting in a late stage inhibition of cancer cell proliferation (78). A reduction in estrogen receptors also increases TGF*B* production, further reducing cancer cell proliferation and complimenting tamoxifen treatment.

Melatonin has recently been shown to increase the antitumor activity of interleukin 2 (IL-2)(125). IL-2, produced by activated T cells, orchestrates the immune response against solid tumors. IL-2 does not suppress the growth of all types of solid tumors. Lung cancer and hepatocarcinoma do not regress with IL-2 activity alone. High levels of melatonin supplements increase the efficiency of IL-2 and produce regression in these resistant forms of cancer (32). Melatonin may produce this amplification of IL-2 antitumor activity by increasing the sensitivity of cancer cells to cytolysis induced by IL-2 lymphocytes (125). Another possible mode of action is an increase in immune lymphocyte and eosinophil numbers (125). This is achieved through melatonin's stimulation of type 2 T-helper cells, producing increases in IL4 and IL5. Melatonin also appears to increase macrophage-mediated suppression without diminishing the tumor necrosis factor (TNF) production (125). Macrophages and fibroblasts are stimulated by melatonin to increase colony stimulating activity.

Another possibility for the increase in autoimmune disease in the elderly is a change in protein production with age (92). The proteins produced function similarly to proteins of youth, but they may have slightly different structures promoting an immune response. This possibility is related to DNA damage increasing with age, rather than immune system regression. It is unlikely to be the exclusive reason for an increase in autoimmune disease with age, but it is a contributing factor.

Melatonin's ability to alleviate immunodepression caused by acute stress, glucocorticoids or cancer is achieved through T-helper lymphocytes, not opioid peptides (68). Immunodepression is often a cause of the progression of cancer. Melatonin stimulates T-helper cells to produce cytokines, which are integral with melatonin in retaining the delicate balance of the immune system. Melatonin stimulates the release of interleukin-2 and γ-interferon (93). In addition to stimulating cytokine release, melatonin induces mRNA production for interleukin-1 in human monocytes (79). A high-affinity binding site in bone marrow cells, specifically T-helper cells, was found to have a Kd of 346 pM (67). Currently, hematopoietic cytokines and bone marrow transplants are used to alleviate the hematopoietic toxicity of chemotherapy that hampers drug dose levels and the inhibition of cancer growth. Recently, melatonin has been shown to provide the same results with fewer side effects, if any, at a reduced financial cost (67).

These results were shown on mice infected with Lewis lung carcinoma and treated with etoposide. Melatonin protected the leukocytes, platelets and marrow granulocyte/macrophage-colony forming units (GM-CFU) from apoptosis caused by etoposide without a decrease in their anticancer action (67). Melatonin protected the GM-CFUs by inducing additional production of colony-stimulating factors (CSFs) (67). Anti-GM-CSF monoclonal antibodies prevented melatonin from protecting bone marrow cells from etoposide induced apoptosis. Melatonin was more effective at protecting the bone marrow, administered with chemotherapy, than accelerating the recovery of these

bone marrow cells when given after the chemotherapy. This important use of melatonin should be considered and implemented by clinicians.

This protection was solidified when the number of lineage-committed myeloid precursor GMU-CFUs were observed to increase without the increase of pluripotent spleen-colony forming units (S-CFU), with the addition of melatonin. An experiment using athymic nude mice led to the conclusion that melatonin can induce the production of endogenous CSFs, specifically GM-CSF, in T-lymphocytes and not macrophages (67). S-CFU was decreased in number when exposed to melatonin. This is caused by melatonin's stimulation of IL4 production from type 2 T-helper cells and IL4's inhibition of S-CFU (12).

GM-CFU numbers are increased with the addition of melatonin, beginning at melatonin's physiological concentration of 0.1 nM. GM-CFU is produced by both T cells and stromal cells (37). When stromal cells were separated from T cells by adherence, melatonin had no affect on GM-CFU production in either T cells or stromal cells. This led to the conclusion that melatonin must stimulate the release of IL4 from type 2 helper cells, which then acts on adherent stromal cells to produce GM-CSF. The addition of anti-IL4 monoclonal antibodies negates the increase in GM-CFU numbers seen with exposure to melatonin. Although, melatonin will not increase GM-CFUs without GM-CSF, even with its stimulation of IL4 production. An additional, yet unknown, signal is needed which may be found in current chemotherapy drugs (121). These drugs are speculated to activate macrophages, possibly producing the needed signal molecule.

Melatonin's regulation of the immune system equilibrium is critical for targeted responses to foreign microorganisms immunopathological disorders, autoimmune diseases, and reproduction (67). Melatonin's influence on the reproductive and immune systems is based on its feedback loops created with type 1 and type 2 T-helper cells. Type 1 T-helper cells (Th1) produce two cytokines related to melatonin stimulation, IL2 and γIFN. Type 2

T-helper cells (Th2) produce two different cytokines related to melatonin stimulation, IL4 and IL5 (28). The Th2 cells posess the high affinity melatonin receptor, and are melatonin's target T-helper cell. At physiological concentrations, melatonin stimulates Th2 cells to produce IL4, which in turn stimulates granulocytes and macrophages to produce colony stimulating factors. *y*-IFN, produced by Th1 cells, inhibits the production of cytokines form Th2 cells and stimulates melatonin production in the pineal gland (28). IL4 inhibits *y*IFN production in Th1 cells (12). Therefore, if IL4 is needed to stimulate GM-CSF, it will also inhibit *y*-IFN production and melatonin production [Fig. 4]. This negative feedback prevents excessive IL4 concentrations. Accordingly, if melatonin levels are low, IL4 will no longer be available to inhibit *y*-IFN production. The subsequent rise in *y*IFN production will increase melatonin production until IL4 levels inhibit *y*IFN production. This basic regulation of melatonin is complicated by the production of melatonin outside the pineal gland and the environmental influence of melatonin production. These factors alter the melatonin plasma concentrations and have an affect on the immune system through the Th2 cells.

Melatonin's stimulation of IL4 production has also been linked to successful pregnancy (67). IL4 has the ability to regulate cell cycle progression, inhibit the growth of human melanomas, enhance the antigenicity of melanoma cells and induce programmed cell death (70). Appropriate IL4 production is essential for cancer prevention.

STRESS AND DISEASE

The hippocampus inhibits the hypothalamic-pituitary-adrenal (HPA) axis. The hippocampus degenerates with age and with neurological impairment. This hippocampal degeneration is hypothesized to be the cause of increased cortisol secretion with age (35). This occurs through a reduction in the sensitivity of steroid feedback. Hippocampal degeneration and age increase and prolong the adrenocortical responses to stress. With chronic duration, adrenocortical responses to stress are a detriment to the body. A slowed reduction of stress related hormones increases the potential for cardiovascular disease, cancer, diabetes, hypertension and weakens the immune system(114).

ACTH concentrations increase with age, compounding the problem of prolonged and increased response to stress. Lack of melatonin, with its ability to inhibit adrenocorticoid secretion, is the reason for elevated ACTH and cortisol plasma concentrations (35). Adrenocorticoid secretion is primarily elevated at night, adding support to melatonin's inhibitory role.

Each individual ages at a different rate. One leading theory to explain this is referred to as the rate of senescence. This theory claims the rate at which an individual ages is influenced by the cumulative exposure to stress and glucocorticoids (17). Glucocorticoids are responsible for hippocampal neuronal degeneration. The hippocampus is responsible for the inhibition of glucocorticoid release. As the hippocampus degenerates, not only is it exposed to higher and more sustained levels of glucocorticoids, its loss of neurons could be responsible for the memory loss seen with senescence (75)

The HPA axis contributes to the normal homeostatic regulation of the body. This axis helps coordinate neuroendocrine and metabolic responses to external and internal

stimuli. Excitement of the HPA axis allows the body to successfully react to short-term stress. The glucocorticoids secreted in response to short-term stress contribute to increased available glucose and lipids for energy and increased blood pressure and suppression of the immune system (114) Chronic exposure to these conditions leads to higher associations with many age-related pathologies. This may be the result of a cumulative exposure to glucocorticoids. As the HPA axis becomes less sensitive to the negative feedback signals required to lower glucocorticoed levels, the prolonged exposure of glucocorticoids may further damage the HPA axis and its negative feedback mechanisms (114).

The HPA axis begins with a signal from the suprahypothalamic brain, resulting from a stress. Hypophysiotropic peptides are released from the hypothalamus, regulating pituitary hormone secretion. These tropic hormones regulate adrenal hormone secretion. These adrenal hormones inhibit the HPA axis to form the negative feedback loop. The hippocampus has one of the highest concentrations of corticosteroid receptors in the brain (114). Thus, the hippocampus may be an important target of feedback inhibition. Decreases in corticosteroid receptors in the hippocampus with age leads to the prolonged recovery from an activated HPA resilience to stressors and increased secretion of ACTH and glucocorticoids (34). When the hippocampus is chronically exposed to high concentrations of glucocorticoids, the glucose transport in hippocampal neurons is disrupted (55). HPA hyperactivity, reduced resilience and high cumulative exposure to glucocorticoids increase the occurance of many age-related diseases, such as atherosclerosis, diabetes and cancer (114). HPA hyperactivity may also lead to impaired immune and cognitive function (54). Research is needed to determine if a correlation between high cumulative glucocorticoid exposure and incidence of Alzheimer disease exists.

High concentrations of cortisol and ACTH are seen in patients suffering from Alzheimer disease. The body is unable to reduce potentially damaging hormone levels as quickly as in youth. The SCN stimulates an increase in early morning glucocorticoid levels. An early morning increase in glucocorticoid concentrations is stimulated by the SCN. This hormone release may help us to wake, and it would likely be linked to circadian cycles by the SCN. Without melatonin for inhibition, recovery from these levels is extended. This chronic exposure to glucocorticoids ages the body and weakens the immune system. Many forms of dementia exhibit disrupted central neurotransmitter function.

Clinical assessments of ageing are based on the endocrine (hormone concentrations) and nervous (sympathetic) systems' integrity. Individual ageing rates vary depending on genetic and environmental influences on systems and tissues. For example, endocrine secretory tissue will vary in mass and cellular composition between each individual, as will hormone secretion rate, distribution space, hormone degradation and excretion rate, sensitivity to feedback regulation, and rhythmic secretory cycles (132).

Most forms of dementia in aged individuals result from errors in protein production. The toxic protein piece that may produce Alzheimer's disease is 42-amino-acid-*B*-amyloid peptide. These peptides build up in the extracellular space, eventually forming plaques. These amyloid plaques toxically effect neurons by disrupting calcium equilibriums, producing oxygen free radicals and facilitating the aggregation of microglia and inflammation (38). Although amyloid plaques are not related to the severity of the dementia, the amyloid plaques may be an initial step, producing a cascade of events, culminating in clusters of neurofibrillary tangles formed with abnormal proteins, eventually producing severe dementia (38). These tau proteins disrupt microtubule function in neurons and are relative to the severity of dementia. Preventing amyloid plaque deposits may help treat Alzheimer's in its early stages.

Patients with acute intermittent porphyria (AIP), an autosomal dominant disorder disrupting the heme biosynthetic pathway, have reduced levels of plasma melatonin during the day and at night(100). AIP patients also show increased levels of tryptophan and serotonin production(101). Heme arginate treatment of the disease decreases both the serotonin and tryptophan levels characteristic of AIP, but does not raise the melatonin levels. Thus, the heme arginate treatment is reducing the serotonin and tryptophan concentrations by some other means than allowing them to be converted into melatonin. These low levels of melatonin may be the cause of acute, recurrent AIP attacks by desynchronizing patients circadian rhythms, thus increasing the effects of environmental risk factors, such as, upsetting the equilibrium of the sex hormones.

The question concerning why would melatonin levels be lower if its precursors, tryptophan and serotonin, are higher, has yet to be explained. This explanation would help identify a more poignant treatment than those currently used. As AIP attacks cause debilitating loss of function in the peripheral, autonomic or central nervous system, the use of melatonin supplements for the prevention of such attacks or alleviation of symptoms should be explored. It is hypothesized that AIP patients over produce delta-aminobutyric acid(Ala) in the liver(101).

Ala is a heme precursor, also producing the same effects as its structural analogue, GABA, inhibitory, on melatonin production in the pineal gland. The low plasma levels of melatonin are not related to the high tryptophan levels. Rather, they are directly related to a high level of Ala(101). Ala poorly penetrates the blood brain barrier, but would readily effect pinealocytes as the vascularzation of the pineal gland exists outside the barrier(101). The decrease in melatonin is caused by an increase in Ala concentration, not by a lack of hemoproteins(101). Ala blocks NAT activity, preventing the production of melatonin and the pinealoctye stimulatory response to NE(101).

Ala causes the low levels of melatonin, suggesting that environmental risk factors, and their related symptoms, are increased by low levels of circulating melatonin and not the cause of these low levels. Until it is determined if Ala overproduction is the exclusive cause of AIP, melatonin supplements may decrease the frequency and severity of acute AIP attacks.

CONCLUSION

The retina relays photoperiod information to the SCN. This information is used to increase melatonin production at night and during the winter months. Vasopressin cells increase in concentration during winter months, increasing melatonin production, storage and secretion. The hypothalamus influences gonadal steroid secretion via the pituitary gland, also signalling the pineal gland. Gonadal steroids have receptors in the pineal gland (influencing melatonin secretion) and in the hypothalamic- pituitary axis (influencing gonadotropin release). Gonadotropins have receptors in the pineal gland. Melatonin significantly influences gonadotropin secretion (and, indirectly, gonadal steroid release) via modifications in LHRH and GnRH release. This complex feedback loop centers melatonin in the endocrine hormonal homeostasis of the body.

Melatonin's progonadal or antigonadal actions depend on photoperiod, age and species. These modifications allow melatonin to regulate reproduction in seasonally breeding mammals by integrating photoperiod into the reproductive physiology.

Conducting studies to determine if melatonin, supplemented to maintain an in vivo concentration similar to youth, extends average age spans in humans is problematic. Humans have relatively long life spans and the studies tracking individuals for many years are filled with unaccountable variables. *Drosophila* experiments have shown decreased life spans when their 24 hour days, based on light and dark cycles, are either increased or decreased to 27 and 21 hours respectively (134). Therefore, melatonin supplements may increase the average age of humans, but more importantly, melatonin supplements increase the quality of life in aged individuals.

So why is melatonin not prescribed by geriatricians on a widespread basis? The answer may lie in the fact that this prescription would require an off-label use. Doctors are often reluctant to prescribe drugs in a manner not precisely approved by the FDA, even if the use is supported by studies in multiple medical journals. Furthermore, the media is apt to follow this conservative decision, resulting in a public that is not given additional options, including the knowledge to ask their doctors questions. Obviously, this lack of medical education of the public is changing rapidly. In the near future, maybe taking your daily melatonin dose will be a common occurrence for almost everyone older than forty-five.

From a geneticists viewpoint, every system in an organism is created to maximize reproductive viability at precisely the same time. Animals with a shorter life span become reproductively active at a comparatively faster rate. Organisms' reproductive systems reach maturity based on their endocrine system's time table. Gonadotropic hormones are regulated by the hypothalamus. The SCN, the internal clock of the body, is directly linked to the retina. Day and night cycles make an organism's biology aware of time. These circadian rhythms direct an organism's reproduction through the tropic hormone melatonin.

Melatonin influences the ageing process indirectly through the reproductive system. When an organism has aged past prime reproductive viability, melatonin levels have already begun a gradual decline continuing until death. Most organisms live long enough to have multiple offspring or protect and raise the few offspring they have. Both strategies increase the potential for continuation of their DNA, and both require melatonin for survival. Animals do not have predestined times of death. Rather, the chance of death increases proportionally with age and inversely with melatonin production.

The current theory of death through old age is the cumulative damage free radicals exert on the body and DNA. Animals consume anti-oxidants from the environment to

prevent this damage. They also produce an anti-oxidant, melatonin. Organisms succumb to disease, cancer, viral and bacterial infections. Melatonin maintains a healthy, vibrant immune system. Melatonin links the immune system with the reproductive system. It protects an organism from death until it can produce offspring. The pineal gland and SCN are the biological clocks of the body. Melatonin is their administrator. Each is scheduled around the only time table important to any organism in an evolutionary sense, the reproductive system. Supplementing melatonin could increase the mean age of a population. It will increase the quality of life of the aged. It will prevent many diseases. It is a valuable preventive tool for medicine.

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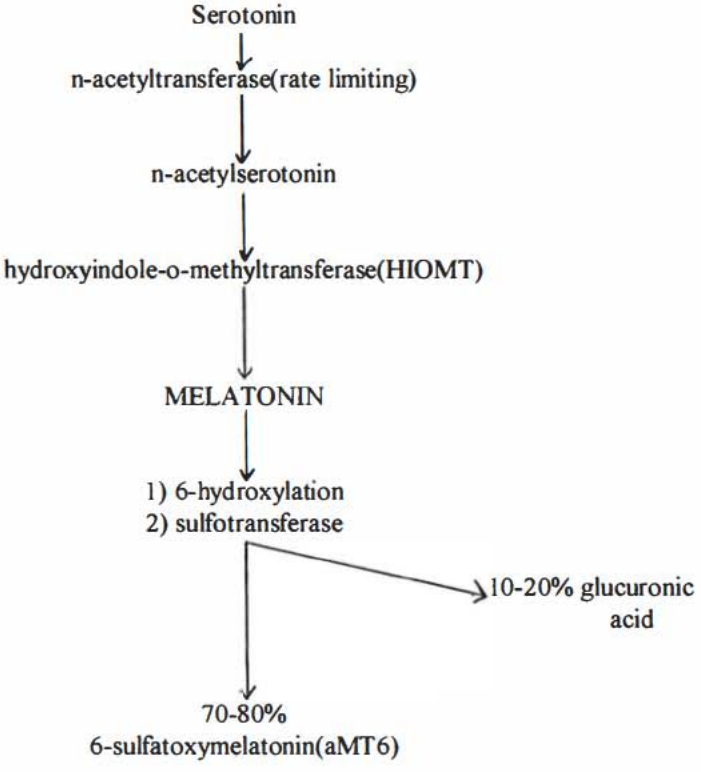

Serotonin
n-acetyltransferase(rate limiting)
n-acetylserotonin
hydroxyindole-o-methyltransferase(HIOMT)
MELATONIN
1) 6-hydroxylation
2) sulfotransferase
10-20% glucuronic acid
70-80%
6-sulfatoxymelatonin(aMT6)

Figure 1

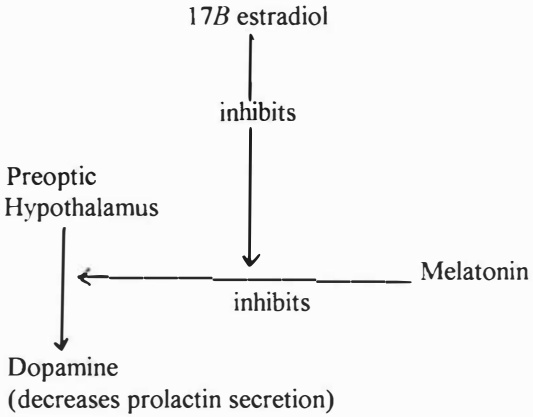
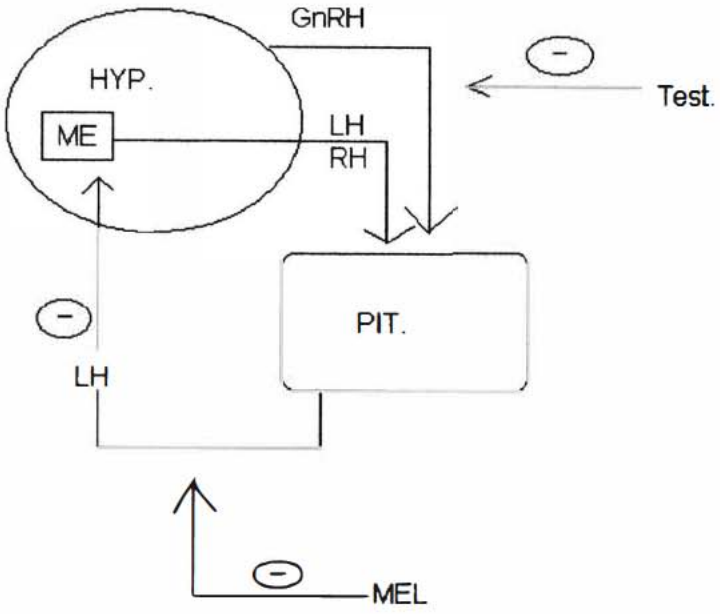

17B estradiol
inhibits
Preoptic
Hypothalamus
Melatonin
inhibits
Dopamine
(decreases prolactin secretion)

Figure 2



FIGURE

HYP - hypothalamus
MEL - melatonin
PIT - pituitary
Test - testosterone

Figure 3

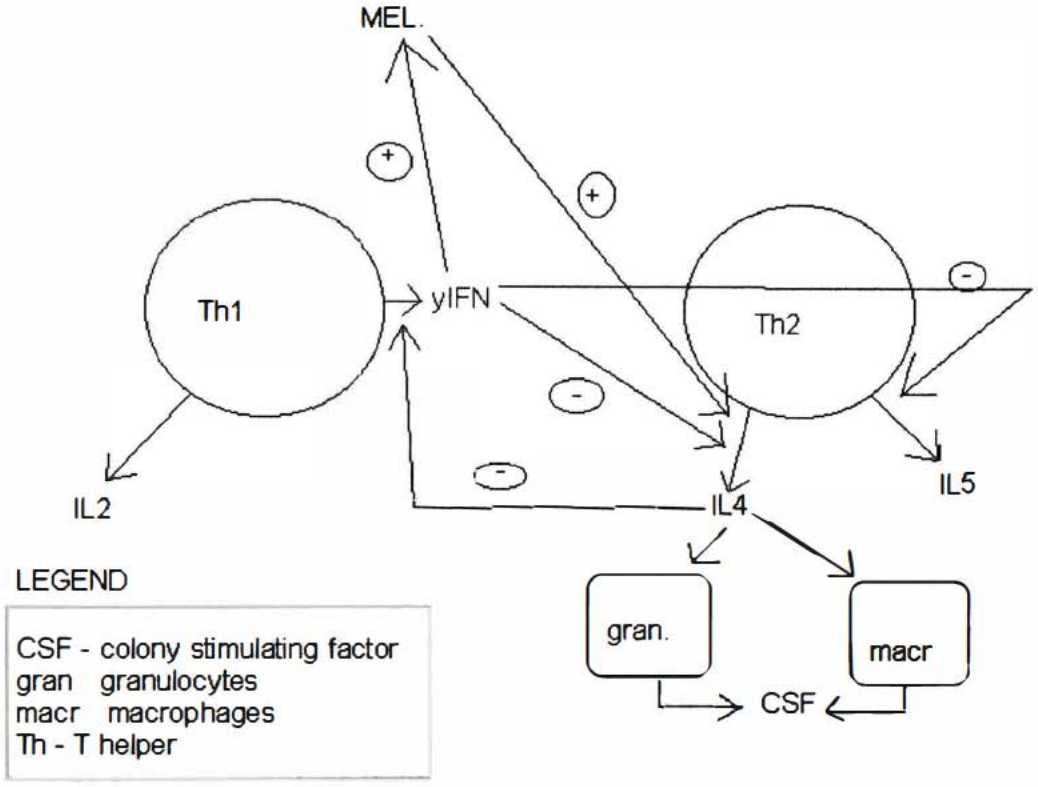

MEL.
+
+
Th1
γIFN
Th2
-
-
-
IL2
IL4
IL5
gran.
macr
CSF
LEGEND
CSF - colony stimulating factor
gran granulocytes
macr macrophages
Th - T helper

Figure 4

VITA

[redacted]